

A Micro-injection System

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SUMMARY

The apparatus works by the thermal expansion of water. The water is contained in a fine, thick-walled capillary tube, one end of which is made into a micro-pipette. The other extremity, which is closed, is heated over varying lengths by warm water in order to obtain the required change in the volume of the water by thermal expansion.

MICRO-INJECTION, as it is used at present, is a modification of the ordinary procedure of injection, in which a reservoir connected to a micro-pipette is used. The necessary increase in pressure is effected by a decrease in capacity (Chambers and Kopac, 1950). In all the current methods the ratio between the capacity of the reservoir and the micro-pipette is very large, and this entails difficulties in their operation. Pierre de Fonbrune (1935) has come nearest to the ideal small-capacity micro-injector. His *injecteur thermique* consists of a short tube completely filled with water. Micro-injections are made by heating the tube electrically so that the water expands. Unfortunately, external temperature-changes strongly affect the system.

The system in this paper is based on the thermal principle of de Fonbrune. Fig. 1 represents a de Fonbrune micromanipulator, on which a 'thermal injector' has been mounted. The tip of the latter extends into the oil chamber in which micro-operations are performed. The injector is essentially a bent, thick-walled capillary tube. The tail of the tube is heated by immersion in a test-tube of warm water. The simplest way to do this is to slide the test-tube on a slanting support, but the same effect can be obtained by changing the level of the water by means of a syringe. The syringe is mounted on a support on the floor and is operated by rotating a circular foot-rest, the piston being moved in one direction by a pulley worked by the operator's feet, and in the other direction by a spring (fig. 2). The heater, which is immersed in the test-tube to keep the water at a fairly constant temperature, is not represented in fig. 1.

The method of making the injector is illustrated in fig. 3.

1. A capillary tube is drawn from a thick-walled glass tube and filled with boiled distilled water (A in fig. 3).
2. The tube is sealed at one end in a small gas flame; at the other end a little water is boiled out (B), after which this extremity is also sealed (C).
3. The tube is heated locally, so that the vapour pressure blows a bubble (D).

4. The bubble is drawn out (E).
5. The thin-walled section is made into a hook, and a bend is made in the thick-walled shaft (F).
6. The whole capillary is refilled by dipping the hook in water and heating the tail.

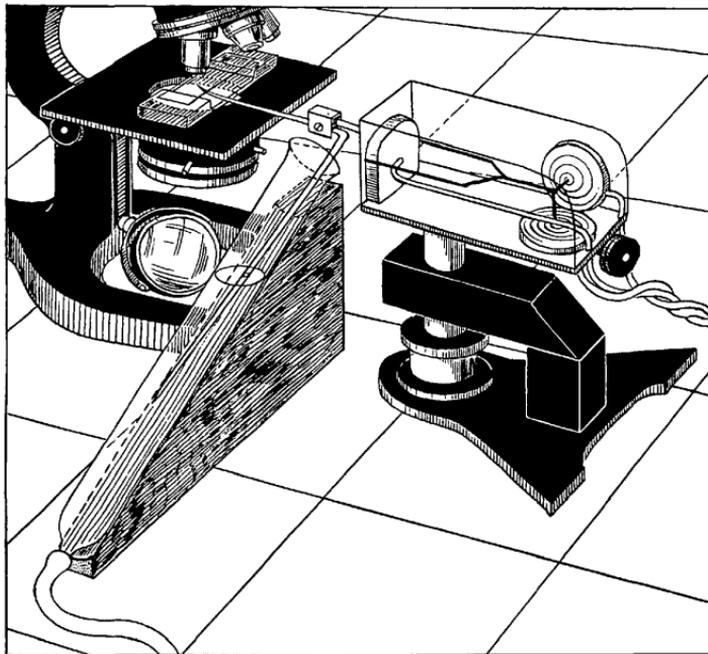


FIG. 1. The micro-manipulator, injector, and test-tube, in position in front of the microscope.

7. The micro-tip proper is made on the de Fonbrune micro-forge (de Fonbrune, 1950), the pulling force being provided by a small weight attached to the hook (G, H; the filament and capillary are shown in the field of the forge). The tube is drawn out, the finished micro-pipette containing a small air gap (I).
8. The air-bubble is replaced by paraffin oil after mounting on the micro-manipulator.

This micro-injecting system has the following properties:

1. The capacity is small.
2. The system offers possibilities for quantitative injections, as there are no air gaps and the amount of expanding water is directly proportional to the length of the tail heated and to the rise in temperature.

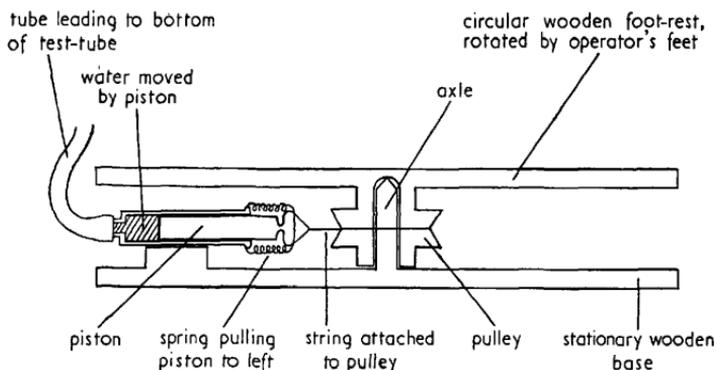


FIG. 2. The apparatus by which the level of the water in the test-tube is controlled (diagrammatic vertical section).

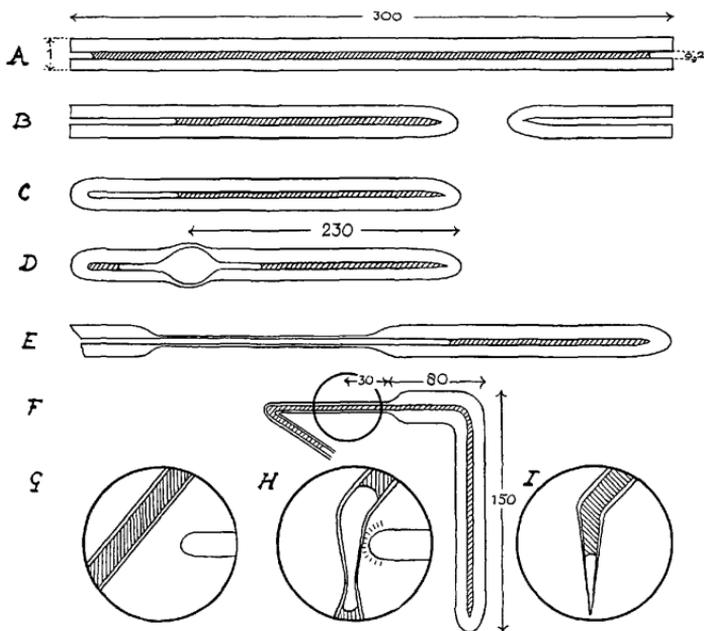


FIG. 3. The diagrams illustrate the method by which the injector is made. For description see text. The numerals represent measurements in millimetres.

3. The force of the expanding water partially eliminates the trouble of clogging tips.
4. Pressure-control is accurate and the pressure-gradient is favourable.
5. The thick wall serves as a thermal insulator for the water in the capillary. Thus external temperature-oscillations are decreased and the pipette 'stays put'.
6. The response is immediate.

Besides injecting, the system is equally suitable for micro-aspirations such as slow draining, the speed of drainage being regulated by controlling the speed of cooling. It should also be useful in picking up single bacteria, as the water never rushes back into the pipette. Finally, as the injection pressure is high, injections could be made into rigid-walled plant-cells.

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